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MICROSCOPY.¹**Orienting Small Objects for Sectioning, and "Fixing" them, when Mounted in Cells.**

I. In one of the recent "Contributions from the Zoological Laboratory of the Museum of Comp. Zoology," Vol. XXV, No. 3, Dr. W. McM. Woodworth describes a method of orienting small objects for the microtome. His method was developed, he states, from one first used by myself. To avoid any misunderstanding, I will say that in answer to a letter from my friend Dr. Woodworth, asking permission to use or describe my method, I replied that he was at liberty to make what use of it he saw fit, or words to that effect. I refer to the subject here, partly because Dr. Woodworth does not state what the original method was, or how he has modified or added to it, but mainly because I believe the original method is much simpler and better adapted to the purpose than his.

My method, which is especially useful when one desires to orient accurately large numbers of small and similar objects, is as follows:

Small strips of glazed writing paper marked with two sets of raised parallel lines running at right angles to each other are cut, and at suitable intervals a very small drop of thick collodion and clove oil, about the consistency of thick honey, is added. The drops are arranged close together along one of the ribs that run lengthwise of the paper. The object to be imbedded is cleared in clove oil, or oil of bergamot—not turpentine. The latter dries too quickly, so that air bubbles are likely to form in the object; and besides it does not mix readily, as it should, with the thick collodion. It is then raised on the point of a knife, and after the excess of oil is drawn off, transferred to a drop of the thick collodion. It may then be adjusted at leisure under the compound or the dissecting microscope, and will stay in any desired position.

When half a dozen or more objects are oriented in reference to the cross lines (which are to be parallel to the section planes) the whole thing is placed in turpentine. This washes out the clove oil and fixes the objects very firmly to the paper. When submerged in turpentine, if desirable, the relation of each object to the orienting lines can be redetermined under the compound microscope with greater precision than before. If any one of them has been inaccurately placed, it may still be moved to some extent, but it is better to note the fact, and

¹Edited by C. O. Whitman, Chicago University.

make the necessary deviations from the section lines when that particular object is sectioned.

The paper with the attached objects is now placed in the paraffine bath, and finally removed and covered with paraffine in the usual way. After cooling in water, the block is trimmed and the softened paper peeled off, leaving the objects in the paraffine, close to the under surface of the block. This surface is now marked by the orienting lines of the ribbed paper and also by the record numbers, which, before imbedding, were written with a soft pencil on the paper. The block is now fixed in the microtome, and the objects cut one after the other, as though a single object had been imbedded; or a number of them may be cut together, if they have been arranged with that object in view. For example, we may use a thinner collodion, and arrange a large number of insect embryos, or small worms in a compact bundle, like a package of cigarettes, and cut them all at once.

Although I have not tried Dr. Woodworth's method, it seems to me that he has merely added to what is described above, several complications, which might in most cases be omitted. He gums the paper to a glass slide, dries it, covers the exposed surface first with a layer of gum and then with a collodion film, each of which must dry separately. The objects cleared in turpentine are then placed in position in the film which is softened and rendered adhesive by exposure to ether vapor, then slide and all are placed in the paraffine bath. Finally after imbedding, the slide is soaked in water to free it from the paper and the paper from the paraffine. In most cases I find it quite unnecessary to gum the paper, as it comes away from the collodion and the paraffine very well without it. It is, moreover, very inconvenient and unnecessary to imbed the paper attached to a glass slide in the paraffine bath. The paper alone can be handled with perfect ease, and it does not curl up or warp in the bath. If any warping occurs, I should say it was due, for obvious reasons, to the use of a collodion film in place of minute drops of collodion and clove oil. I should suppose also that any object of considerable size, say the egg of *Limulus*, could not be easily fixed in the manner suggested by Dr. Woodworth, for it is merely the adhesiveness of the small amount of turpentine on the object which must be depended upon to hold it in place. But as the turpentine evaporates rapidly, this would tend to free the object, or else fill it with air bubbles before the requisite number could be oriented, preparatory to softening the collodion in the ether vapor.

The advantages of the method, as I use it, are many; ease, rapidity (although we need not hurry) and accuracy of orientation; time saved

in imbedding and sectioning a considerable number of objects as one; and above all when many objects much alike are to be imbedded, there is no danger of confusion, since each one is plainly marked with its appropriate number.

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II. As every one knows, it is a great nuisance to mount under one cover, a large number of objects that tend to roll about into undesirable positions. It is often necessary to mount each one separately and then roll it about at great risk, till it is just where we want it. And after all it is impossible to roll some things into place. I have used a modification of the method described above in mounting large numbers of objects under one cover, in perfect order, and in any desired position.

In mounting the eggs of *Limulus*, or heads of insect embryos, etc., I construct a cell of the requisite dimensions, and place in it small drops, close together in rows, of the thick collodion and clove oil. An egg is taken out of the clove oil, drained, and placed in a drop of collodion in the desired position. A great many eggs may thus be arranged like serial sections under one cover glass. Before adding the balsam, the slide is immersed in turpentine, which serves to wash away the clove oil and leave the eggs firmly fixed in the collodion.

The only precaution necessary is not to use too much collodion. It is surprising to find the small amount necessary, and the firmness with which the objects are held by it in place.

I have recently used, with a class of beginners, the above method of imbedding, with satisfactory results—merely as a matter of convenience in manipulating small objects easily soiled or broken in handling. Any glazed paper, or glazed tracing cloth will do, provided the collodion and clove oil is thick enough. The raised ribs may be replaced by fine black lines drawn with a soft pencil. These lines like the numbers are transferred to the paraffine when the paper is removed.

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